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Assessment of genetically modified soybean MON 87751 for food and feed uses under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2014-121)

EFSA Panel on Genetically Modified Organisms (GMO), Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikołaj Antoni Gralak, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli, Jean-Michel Wal, Fernando Álvarez, Michele Ardizzone, Antonio Fernandez Dumont, José Ángel Gómez Ruiz, Nikoletta Papadopoulou and Konstantinos Paraskevopoulos

Abstract

Soybean MON 87751 was developed through *Agrobacterium tumefaciens*-mediated transformation to provide protection certain specific lepidopteran pests by the expression of the Cry1A.105 and Cry2Ab2 proteins derived from *Bacillus thuringiensis*. The molecular characterisation data and bioinformatic analyses did not identify issues requiring assessment for food and feed safety. None of the compositional, agronomic and phenotypic differences identified between soybean MON 87751 and the conventional counterpart required further assessment. The GMO Panel did not identify safety concerns regarding the toxicity and allergenicity of the Cry1A.105 and Cry2Ab2 proteins as expressed in soybean MON 87751, and found no evidence that the genetic modification might significantly change the overall allergenicity of soybean MON 87751. The nutritional impact of soybean MON 87751-derived food and feed is expected to be the same as those derived from the conventional counterpart and non-GM commercial reference varieties. The GMO Panel concludes that soybean MON 87751, as described in this application, is nutritionally equivalent to and as safe as the conventional counterpart and the non-GM soybean reference varieties tested, and no post-market monitoring of food and feed is considered necessary. In the case of accidental release of viable soybean MON 87751 seeds into the environment, soybean MON 87751 would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of soybean MON 87751. In conclusion, soybean MON 87751, as described in this application, is as safe as its conventional counterpart and the tested non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment.

Keywords: GMO, soybean (*Glycine max*), MON 87751, Cry1A.105, Cry2Ab2, insect resistant, Regulation (EC) No 1829/2003

Requestor: Competent Authority of the Netherlands

Question number: EFSA-Q-2014-00719

Correspondence: GMO@efsa.europa.eu

Panel members: Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikolaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Hanspeter Naegeli, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli and Jean-Michel Wal.

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Summary

Following the submission of application EFSA-GMO-NL-2014-121 under Regulation (EC) No 1829/2003 from Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) was asked to deliver a scientific opinion on the safety of the genetically modified (GM) soybean (*Glycine max* (L.) Merr.) MON 87751 (Unique Identifier MON-87751-7). The scope of application EFSA-GMO-NL-2014-121 is for import and processing, and food and feed uses of soybean MON 87751 within the European Union (EU), but excludes cultivation in the EU.

The GMO Panel evaluated soybean MON 87751 with reference to the scope and appropriate principles described in Regulation (EU) 503/2013 and its guidelines for the risk assessment of GM plants. The evaluation addressed the following components of the risk assessment: the molecular characterisation of the inserted DNA and analysis of the expression of the corresponding proteins; the comparative analyses of compositional, agronomic and phenotypic characteristics; the safety of the newly expressed proteins and of the whole food and feed with respect to potential toxicity, allergenicity and nutritional characteristics; the environmental risk assessment and the post-market environmental monitoring (PMEM) plan.

The molecular characterisation data establish that soybean MON 87751 contains a single insert consisting of one copy of the *cry1A.105* and *cry2Ab2* expression cassettes. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other open reading frames present within the insert or spanning the junctions between the insert and genomic DNA, do not indicate significant similarities to toxins and allergens. The stability of the inserted DNA and of the introduced insect resistance trait is confirmed over several generations. The Cry1A.105 and Cry2Ab2 proteins were expressed and the methodology used to quantify their levels is considered adequate. The protein characterisation data comparing the structural, biochemical and functional properties of plant- and microbe-derived Cry1A.105 and Cry2Ab2 proteins indicate that these proteins are equivalent and the microbe-derived proteins can be used in the safety studies.

The comparative assessment of compositional, agronomic and phenotypic characteristics does not identify differences between soybean MON 88751 and its conventional counterpart requiring further assessment. The GMO Panel did not identify safety concerns regarding the toxicity and allergenicity of the Cry1A.105 and Cry2Ab2 proteins, as expressed in soybean MON 87751 and found no evidence that the genetic modification might significantly change the overall allergenicity of soybean MON 87751. The nutritional impact of soybean MON 87751-derived food and feed is expected to be the same as those derived from the conventional counterpart and non-GM commercial reference varieties. The GMO Panel concludes that soybean MON 87751, as described in this application, is nutritionally equivalent to and as safe as the conventional counterpart and the non-GM soybean reference varieties tested, and no post-market monitoring of food and feed is considered necessary.

Considering the introduced traits, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that soybean MON 87751 would not raise safety concerns in the event of accidental release of viable GM soybean seeds into the environment. The PMEM plan and reporting intervals are in line with the intended uses of soybean MON 87751.

The literature searches did not identify any relevant publications. In the context of PMEM, the applicant should improve future literature searches according to the GMO Panel recommendations.

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-NL-2014-121, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. The GMO Panel concludes that soybean MON 87751, as described in this application, is as safe as its conventional counterpart and the tested non-GM soybean reference varieties with respect to potential effects on human and animal health, and the environment.

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1. Introduction

The scope of application EFSA-GMO-NL-2014-121 is for food and feed uses, import and processing of soybean MON 87751 and does not include cultivation in the European Union (EU).

Soybean MON 87751 was developed to confer resistance against certain lepidopteran pests. This is achieved by the expression of the *cry1A.105* and *cry2Ab2* genes from *Bacillus thuringiensis (Bt)*.[1]

1.1. Background

On 8 October 2014, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application EFSA-GMO-NL-2014-121 for authorisation of soybean MON 87751 (Unique Identifier MON-87751-7), submitted by Monsanto Europe (hereafter referred to as the applicant) within the framework of Regulation (EC) No 1829/2003 on GM food and feed.[2]

After receiving application EFSA-GMO-NL-2014-121, and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the application available to the public on the EFSA website.[3] EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 22 December 2014, EFSA received additional information requested under completeness check on 25 November 2014. On 22 January 2015, EFSA declared the application valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[4] following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003 to request their scientific opinion. Member States had three months after the opening of the Member State commenting period (until 4 July 2015) to make their opinion known.

The GMO Panel requested additional information from the applicant on 29 January 2015 (EURL-JRC), 31 March 2015, 5 June 2015, 2 December 2015, 23 December 2015, 11 February 2016, 23 February 2016, 18 March 2016, 23 May 2016, 20 July 2016, 7 December 2016, 23 December 2016, 10 February 2017, 8 March 2017, 14 March 2017, 3 May 2017, 6 July 2017, 2 August 2017, 25 September 2017, 23 November 2017, 4 December 2017 and 7 March 2018. EFSA received the requested information on 12 March 2015 (EURL-JRC), 1 July 2015, 15 July 2015, 9 December 2015, 8 February 2016, 24 February 2016 and 14 March 2016, 22 March 2016, 29 March 2016, 21 July 2016, 22 September 2016, 20 December 2016, 23 December 2016, 11 April 2017, 15 May 2017, 19 May 2017, 31 May 2017, 14 August 2017, 22 August 2017, 23 November 2017, 4 December 2017, 30 January 2018 and 3 May 2018, respectively. The applicant also spontaneously submitted additional information on 20 April 2016, 2 May 2017, 5 October 2017 and 22 December 2017.

In the context of the contracts OC/EFSA/GMO/2013/01 and OC/EFSA/GMO/2014/01, contractors performed preparatory work and delivered reports on the methods applied by the applicant in performing bioinformatic and statistical analyses, respectively.

In giving its scientific opinion on soybean MON 87751 to the European Commission, Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of 6 months from the acknowledgement of the valid application. As additional information was requested by the GMO Panel, the time limit of six months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, this Scientific Opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5).

1.2. Terms of Reference as provided by the requestor

The GMO Panel was requested to carry out a scientific risk assessment of soybean MON 87751 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

[1] Dossier Part II – Section 1.2.2.1.

[2] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

[3] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2014-00719

[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.03.2001, p. 1–38.

Where applicable, any conditions or restrictions for the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food and feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

The GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the GMO Panel did not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

2. Data and methodologies

2.1. Data

In delivering its Scientific Opinion, the GMO Panel took into account application EFSA-GMO-NL-2014-121, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2.2. Methodologies

The GMO Panel carried out a scientific risk assessment of soybean MON 87751 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003.

The GMO Panel took into account Regulation (EU) No 503/2013 and the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA GMO Panel, 2011a), the environmental risk assessment (ERA) of GM plants (EFSA GMO Panel, 2010a) and the post-market environmental monitoring (PMEM) of GM plants (EFSA GMO Panel, 2011b).

The GMO Panel took into account the criteria included in the EFSA Scientific Committee (2011) guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food and feed for GMO risk assessment' and in the 'Explanatory statement for its applicability (EFSA, 2014), to perform the assessment of the 90-day feeding studies provided.

The GMO Panel also assessed the applicant's literature searches, which include a scoping review, in accordance with the recommendations on literature searching outlined in EFSA (2010, 2017).

The comments raised by Member States are addressed in Annex G of EFSA's overall opinion[5] and were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Systematic literature review[6]

The GMO Panel assessed the applicant's literature searches on soybean MON 87751, which include a scoping review, according to the guidelines given in EFSA (2010, 2017).

A systematic literature review as referred to in Regulation (EU) No 503/2013 has not been provided in support of the risk assessment of application EFSA-GMO-NL-2014-121. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value in undertaking a systematic review for soybean MON 87751 at present.

Although the overall quality of the performed literature searches is acceptable, the GMO Panel considers that future searches on soybean MON 87751 could be improved. The GMO Panel therefore recommends the applicant to:

- ensure that enough search term variation is used (covering possible synonyms, related terms, acronyms, spelling variants, old and new terminology, brand and generic names, lay and scientific terminology, common typos, translation issues);
- use truncation consistently;
- include controlled vocabulary (subject indexing) in the searches when available, and where subject headings are available use both free-text terms and controlled vocabulary in the searches;

[5] Available online: http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2018-00505
[6] Dossier: Part II – Section 7; additional information: 8/2/2016, 20/12/2016 and 22/12/2017.

- adapt the search to the size of the identified publications (and thus not combine search sets when one of the search sets already yields only a small number of publications);
- assess the relevance and risk assessment implications of publications retrieved via searches beyond electronic bibliographic databases.

The literature searches did not identify any relevant publications on soybean MON 87751.

3.2. Molecular characterisation

3.2.1. Transformation process and vector constructs[7]

Soybean MON 87751 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation of meristem tissues derived from germinated soybean seeds (*Glycine max* (L.) Merr.) using a disarmed strain AB30 containing the PV-GMIR13196 vector. The PV-GMIR13196 vector contained two T-DNAs, T-DNA I with the *cry2Ab2* and *cry1A.105* expression cassettes between the right and left borders which confer insect resistance. T-DNA II contained the *aadA* and *splA* expression cassettes between the right and left borders, which were used for the selection of transformed plants. Although both T-DNAs were initially inserted during transformation, they were not genetically linked and T-DNA II was eliminated by crossing, therefore only T-DNA I is present in soybean MON 87751 and is described below.

The *cry1A.105* expression cassette contains the following genetic elements: the promoter, leader and chloroplast targeting sequences from an *Arabidopsis thaliana rbcS* gene family encoding the small subunit ats1A; the codon-optimised *cry1Ab*, *cry1F* and *cry1Ac* coding sequences assembled to produce the Cry1A.105 chimeric protein, and the 3' untranslated region of the *pt1* gene from *Medicago truncatula.*

The *cry2Ab2* expression cassette contains the following genetic elements: the promoter, leader and intron sequences from the *act2* gene from *A. thaliana;* the *CTP2* chloroplast targeting sequence of the *shkG* gene from *A. thaliana;* the codon-optimised *cry2Ab2* gene from *B. thuringiensis*; and the 3' untranslated region of the *mt* gene from *Oryza sativa*.

The vector backbone contains elements necessary for the maintenance and selection of the plasmid in bacteria.

3.2.2. Transgene constructs in the GM plant

Molecular characterisation of soybean MON 87751 was performed by next generation sequencing (NGS) and junction sequence analysis (JSA), polymerase chain reaction (PCR) and DNA sequence analysis in order to determine insert copy number, size and organisation of the inserted sequences, and to confirm the absence of plasmid backbone sequences.[8] The approach used is acceptable in terms of coverage and sensitivity.

NGS/JSA of the whole genome indicates that soybean event MON 87751 contains a single insert, which consists of a single copy of T-DNA I in the same configuration as in the PV-GMIR13196 transformation vector. NGS/JSA also indicates the absence of vector backbone and T-DNA II sequences.

The nucleotide sequence of the entire insert of soybean MON 87751 together with 1334 bp of the 5' and 1187 bp of the 3' flanking regions were determined. The results are in line with those shown by the NGS/JSA analyses. The insert of 10119 bp is identical to the T-DNA I of PV-GMIR13196. A comparison of the flanking regions sequences with the pre-insertion locus indicates a 1 bp insertion and a 7 bp deletion at the insertion site as well as a 16 bp deletion in the 5' flanking region. The possible interruption of known endogenous soybean genes by the insertion in event MON 87751 was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert.[9,10] The results of these analyses do not indicate the interruption of any known endogenous genes in soybean MON 87751.

The results of segregation (see below) and bioinformatic analyses establish that the insert is located in the nuclear genome.

[7] Dossier: Part II – Section 1.2.1.
[8] Dossier: Part II – Section 1.2.2.2; additional information: 29/3/2016.
[9] Dossier Part II – Section 1.2.2.2(e).
[10] Additional information: 9/12/2015 and 19/5/2017.

Updated bioinformatic analyses of the amino acid sequences of the newly expressed Cry1A.105 and Cry2Ab2 proteins reveals no significant similarities to known toxins and allergens.[9] In addition, updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert or spanning the junctions between the insert and genomic DNA do not indicate significant similarities to toxins and allergens.[9,10]

In order to assess the possibility for horizontal gene transfer by homologous recombination, the applicant performed a sequence identity analysis of the inserted regions of bacterial origin in soybean MON 87751.[10,11] The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.5.1.2.

3.2.3. Protein characterisation and equivalence

Soybean MON 87751 expresses two new proteins, Cry1A.105 and Cry2Ab2.

Given the technical restraints in producing large enough quantities for safety testing from plants, these proteins were recombinantly produced in *Escherichia coli.* Prior to safety studies, a set of biochemical methods was employed to demonstrate the equivalence between soybean- and microbe-derived proteins. Purified proteins from these sources were characterised and compared in terms of their physicochemical, structural and functional properties.

Cry1A.105 characterisation and equivalence.[12] Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis show that plant- and microbe-derived Cry1A.105 proteins have the expected molecular weight of ~133 kDa and are comparably immunoreactive to Cry1A.105 protein specific antibodies. Glycosylation detection analysis demonstrates that none of the Cry1A.105 proteins were glycosylated. Amino acid sequence analysis by mass spectrometry methods shows that both proteins match the deduced sequence as defined by the *cry1A.105* gene. In addition, N-terminal sequencing analysis shows that the N-terminus of the soybean-derived Cry1A.105 protein is intact and four amino acids originating from the chloroplast transit peptide (CTP) are also present. These data are consistent with the microbe-derived Cry1A.105 protein, designed to contain also the four amino acids from the CTP. Functional equivalence was demonstrated by an insect feeding bioassay which shows that both proteins have comparable insecticidal activity.

Cry2Ab2 characterisation and equivalence.[13] SDS-PAGE and western blot analysis show that plant- and microbe-derived Cry2Ab2 proteins have the expected molecular weight of ~62 kDa and are comparably immunoreactive to Cry2Ab2 protein specific antibodies. Glycosylation detection analysis demonstrates that none of the Cry2Ab2 proteins were glycosylated. Amino acid sequence analysis by mass spectrometry methods shows that both proteins match the deduced sequence as defined by the inserted *cry2Ab2* gene. In addition, N-terminal sequencing analysis shows that the first 15 amino acids of the N terminus of the soybean-derived Cry2Ab2 protein are truncated. These data are consistent with the microbe-derived Cry2Ab2 protein designed to also start at amino acid 16. Functional equivalence was demonstrated by an insect feeding bioassay which shows that both proteins have comparable insecticidal activity.

The protein characterisation data comparing the structural, biochemical and functional properties of plant- and microbe-derived Cry1A.105 and Cry2Ab2 proteins, indicate that these proteins are equivalent. Therefore, the GMO Panel accepts the use of the Cry1A.105 and Cry2Ab2 proteins produced in bacteria for the safety studies.

3.2.4. Information on the expression of the insert[14]

Protein levels of Cry1A.105 and Cry2Ab2 were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested from replicated field trials across five locations in the USA during the 2012 growing season. Samples analysed included leaves (V3–V4, V5–V7, R2–R3, R4), root (R6), forage (R6), pollen/anther (R2, collected only from one site) and seeds (R8). The mean values, standard deviations and ranges of protein expression levels in seeds and forage of the Cry1A.105 and Cry2Ab2 proteins are summarised in Table 1.

[11] Dossier: Part II – Section 1.2.2.5.
[12] Dossier: Part II – Section 1.4.1.1(a); additional information: 9/12/2015.
[13] Dossier: Part II – Section 1.4.1.1(b).
[14] Dossier: Part II – Section 1.2.2.3.

Table 1: Means, standard deviations and ranges of protein levels in seeds (n = 20) and forage (n = 20) (μg/g dry weight) from soybean MON 87751

Tissue (Developmental stage)	Cry1A.105	Cry2Ab2
Seeds (R8)	2.40[(a)] ± 0.50[(b)] (1.70–3.20)[(c)]	4.0 ± 0.77 (2.60–5.10)
Forage (R6)	230.0 ± 91.0 (110.0–440.0)	14.0 ± 2.20 (11.0–18.0)

(a): Mean.
(b): Standard deviation.
(c): Range.

3.2.5. Inheritance and stability of inserted DNA[15]

Genetic stability of the soybean MON 87751 insert was assessed by NGS/JSA for five generations and PCR-based segregation analysis from three generations. The results indicate that all the plants tested retain the single copy of the insert and flanking regions, which were stably inherited in subsequent generations. The results support the presence of a single insertion, segregating in a Mendelian fashion.

3.2.6. Conclusion on molecular characterisation

The molecular characterisation data establish that soybean MON 87751 contains a single insert consisting of one copy of the *cry1A.105* and *cry2Ab2* expression cassettes. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA do not indicate significant similarities to toxins and allergens. The stability of the inserted DNA and of the introduced insect resistance trait is confirmed over several generations. The Cry1A.105 and Cry2Ab2 proteins are expressed and the methodology used to quantify their levels is considered adequate. The protein characterisation data comparing the structural, biochemical and functional properties of plant- and microbe-derived Cry1A.105 and Cry2Ab2 proteins indicate that these proteins are equivalent and the microbe-derived proteins can be used in the safety studies.

3.3. Comparative analysis

3.3.1. Choice of comparator and production of material for the comparative assessment[16]

Application EFSA-GMO-NL-2014-121 presents data on agronomic and phenotypic characteristics, as well as on forage and seed composition of soybean MON 87751 derived from a field trial study performed at nine sites in the USA in 2012, and from studies performed under environmentally controlled conditions (Table 2).

Table 2: Overview of comparative assessment studies with soybean MON 87751 provided in application EFSA-GMO-NL-2014-121

Study focus	Study details	Comparator	Commercial non-GM soybean reference varieties[(b)]
Agronomic and phenotypic analysis	Field trials, 2012, USA, nine locations[(a)]	A3555	Twenty[(c)]
	Pollen characteristics study under controlled conditions	A3555	Four[(d)]
	Seed germination study under controlled conditions	A3555	Twelve[(e)]

[15] Dossier: Part II – Section 1.2.2.4; additional information: 22/08/2017 and 23/11/2017.
[16] Dossier: Part II – Section 1.3.1 and 1.3.2.1.

Study focus	Study details	Comparator	Commercial non-GM soybean reference varieties[(b)]
Compositional analysis	Field trials, 2012, USA, eight locations[(a)]	A3555	Nineteen[(f)]

Non-GM: non-genetically modified.
(a): Except at one location for the agronomic and phenotypic analysis (Miami, OH) data were obtained from the same field trials.
(b): Four different varieties were grown at each location of the field trials.
(c): Hoffman H419, DWIGHT, Crows C2804, Garst 3585N, Midland 363, Crows C3908, NuPride 3202, C3211N, Midwest Genetics G2712, A3244, Stewart SB3819, Wilken 3316, Hoffman HS387, LG C3540, Stine 3300-0, A3525, Lewis 391, WILLIAMS 82, Stewart SB3454, Crows C37003N.
(d): Garst 3585N, FS 3591, eMerge 348TC, Midland 363.
(e): LG C3540, Crows C37003N, A3244, NuPride 3202, DWIGHT, Crows C2804, Garst 3585N, Midland 363, Stine 3300-0, A3525, Hoffman HS387, Wilken 3316.
(f): DWIGHT, Crows C2804, Garst 3585N, Midland 363, Crows C3908, NuPride 3202, C3211N, Midwest Genetics G2712, A3244, Stewart SB3819, Wilken 3316, Hoffman HS387, LG C3540, Stine 3300-0, A3525, Lewis 391, WILLIAMS 82, Stewart SB3454, Crows C37003N.

The field trial study was conducted in typical soybean growing areas of the USA[17], representing regions of diverse agronomic practices and environmental conditions. At each site, the following materials were grown in a randomised complete block design with four replicates: soybean MON 87751, a non-GM comparator (soybean A3555), and four non-GM soybean reference varieties. All materials were treated (sprayed) with required maintenance pesticides according to local requirements. Depending on the focus of the study, the number of locations from which the data were collected and reference varieties used differs (see Table 2).

The comparator used in the comparative assessment studies was a non-GM soybean variety (A3555) with the same genetic background to that of soybean MON 87751 (as documented by the pedigree), and is considered the conventional counterpart.

3.3.2. Statistical analysis of field trial data[18]

The statistical analysis of the agronomic, phenotypic and compositional data from the 2012 field trial study followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a) and complied with Regulation (EU) No 503/2013. This includes the application of a difference test (between the GM soybean and its conventional counterpart) and an equivalence test (between the GM soybean and the set of non-GM soybean reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[19]

3.3.3. Agronomic and phenotypic analysis[20]

3.3.3.1. Agronomic and phenotypic characteristics tested under field conditions

The agronomic and phenotypic endpoints evaluated in the field trials were: early stand count, days to 50% flowering, plant lodging, pod shattering, plant height, final stand count, seed moisture, 100 seed weight and yield. Visually observable responses to naturally occurring diseases, abiotic stress and arthropod damage were also recorded in order to provide indications of altered stress responses of soybean MON 87751 as compared with its conventional counterpart.

The test of difference shows statistically significant differences between soybean MON 87751 and its conventional counterpart for days to 50% flowering, plant lodging, plant height and seed moisture. The test of equivalence shows that all these endpoints were equivalent to the non-GM soybean reference varieties (equivalence category I).

[17] The field sites were located in Jackson (AR), Story (IA), Jefferson (IA), Champaign (IL), Pawnee (KS), Perquimans (NC), Merrick (NE), Miami (OH) and Lehigh (PA). In the field trial located in Miami only agronomic and phenotypic characteristics were evaluated.
[18] Dossier: Part II – Section 1.3.2.2.
[19] The results of the equivalence test are categorised into four possible outcomes: category I (indicating full equivalence); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).
[20] Dossier: Part II – Section 1.3.5; additional information: 15/7/2015, 24/2/2016, 14/3/2016 and 22/3/2016.

3.3.3.2. Agronomic and phenotypic characteristics tested under controlled conditions

Seed characteristics

The applicant reported data on seed characteristics of soybean MON 87751 (Table 2). Seeds were incubated under controlled conditions at six different temperature regimes. The endpoints analysed were the numbers of normal germinated seeds, abnormal germinated seeds, hard seeds, dead seeds and firm swollen seeds. No statistically significant differences are observed between soybean MON 87751 and its conventional counterpart, except for % normal and abnormal germinated seeds at one temperature regime. For all endpoints and temperature regimes, the observed values in soybean MON 87751 are within the range of those observed in the non-GM reference varieties.

Although the applicant refers to seed dormancy when discussing the generated data on soybean MON 87751 seeds characteristics, no data on induced seed dormancy were supplied. The GMO Panel considers that only the conclusions on germination of F_1 seeds of soybean MON 87751 are substantiated by the provided data.

Pollen characteristics

The applicant reported data on pollen characteristics of soybean MON 87751 (Table 2). The endpoints analysed were pollen diameter and viability via the Alexander stain method. No significant differences between soybean MON 87751 and its conventional counterpart for pollen diameter and stain uptake are observed.

3.3.4. Compositional analysis[21]

Soybean MON 87751 seeds and forage harvested from the field trial study in the USA in 2012 (Table 2) were analysed for 74 different constituents (seven in forage[22] and 67 in seeds[23]), including the key constituents recommended by the OECD (2012). For 14 fatty acids,[24] more than 50% of the observations were below the limit of quantification.

The statistical analysis was applied to the remaining 58 constituents (six in forage and 52 in seeds); a summary of the outcome of the test of difference and the test of equivalence is presented in Table 3.[25]

The combination of the test of difference and the test of equivalence could be applied to the 58 endpoints, with the following results:

For soybean MON 87751, statistically significant differences with the conventional counterpart are identified for 14 endpoints in seeds[26] and three in forage.[27] All the endpoints fall under equivalence categories I or II.

[21] Dossier: Part II – Section 1.3.4; additional information: 15/7/2015.
[22] Crude protein, crude fat, ash, moisture, carbohydrates by calculation, acid detergent fibre (ADF) and neutral detergent fibre (NDF).
[23] Protein, total fat, ash, moisture, carbohydrates, acid detergent fibre (ADF), neutral detergent fibre (NDF), calcium, phosphorus, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), palmitic acid (16:0), palmitoleic acid (16:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), γ-linolenic acid (18:3), arachidic acid (20:0), eicosenoic acid (20:1), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), arachidonic acid (20:4), behenic acid (22:0), α-tocopherol, phylloquinone, daidzein, genistein, glycitein, lectin, phytic acid, raffinose, stachyose, trypsin inhibitor, Gly m 1, Gly m 3, Gly m 4, Gly m 5, Gly m 6, Gly m 8, Gly m Bd 28 K and Gly m Bd 30 K.
[24] Caprylic acid, capric acid, lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, pentadecenoic acid, palmitoleic acid, heptadecanoic acid, heptadecenoic acid, γ-linolenic acid, eicosadienoic, eicosatrienoic acid, and arachidonic acid.
[25] Moisture was measured for seed and forage and used only for conversion of components to dry weight, but were not statistically analysed as such.
[26] Total protein, carbohydrates by calculation, glycine, methionine, proline, calcium, phosphorus, stearic acid, oleic acid, linoleic acid, arachidic acid, behenic acid, phylloquinone and raffinose.
[27] Neutral detergent fibre (NDF), carbohydrates by calculation and total fat.

Table 3: Outcome of the comparative compositional analysis in grains and forage of soybean MON 87751. The table shows the number of endpoints in each category

		Test of difference[a]	
		Not different	**Significantly different**
Test of equivalence[b]	Category I/II	41	17[c]
	Category III/IV	–	–
	Not categorised	–	–
	Total endpoints	58	

(a): Comparison between soybean MON 87751 and its conventional counterpart.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Endpoints with significant differences between soybean MON 87751 and its conventional counterpart falling in equivalence category I-II. For seeds: total fat, carbohydrates by calculation, stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), behenic acid (C22:0), glycine, proline, protein, calcium, phosphorus, raffinose and phylloquinone. For forage: total fat, NDF and carbohydrates by calculation.

The GMO Panel assessed all significant differences between soybean MON 87751 and its conventional counterpart, taking into account the potential impact on the plant metabolism and the natural variability observed for the set of non-GM commercial reference varieties. No endpoints showing significant differences between soybean MON 87751 and its conventional counterpart and falling under category III/IV are identified.

3.3.5. Conclusion on comparative analysis

The GMO Panel concludes that none of the agronomic, phenotypic and compositional changes identified with respect to the conventional counterpart and the non-GM soybean reference varieties need further assessment regarding food and feed safety and their environmental impact.

3.4. Food and feed safety assessment

3.4.1. Effects of processing

Soybean MON 87751 will undergo existing production processes used for conventional soybean. No novel production process is envisaged. Therefore, processing of soybean MON 87751 into food and feed products is not expected to result in products being different from those derived from non-GM varieties.

3.4.2. Influence of temperature and pH on newly expressed proteins

3.4.2.1. Effect of temperature on newly expressed proteins[28]

The thermal stability of the microbe-derived Cry1A.105 protein was evaluated for 15 or 30 min at 25, 37, 55, 75 and 95°C, and the insecticidal activity and integrity of the protein was assessed by an insect feeding bioassay and SDS-PAGE, respectively. Heating at 75°C or above (for 15 or 30 min) results in the complete loss of the Cry1A.105 activity. SDS-PAGE indicates a detectable reduction in the Cry1A.105 band intensity when the protein is heated at 95°C for 30 min.

The thermal stability of the microbe-derived Cry2Ab2 protein was evaluated for 15 or 30 min at 25, 37, 55, 75 and 95°C and the insecticidal activity and integrity of the protein was assessed by an insect feeding bioassay and SDS-PAGE, respectively. Heating at 55°C or above (for 15 or 30 min) results in the complete loss of the Cry2Ab2 activity. SDS-PAGE indicates a significant reduction in the Cry2Ab2 band intensity when the protein is heated at 75 or 95°C for 15 or 30 min.

3.4.2.2. Effect of pH on newly expressed proteins[29]

The effect of pH on the intactness of the microbe-derived Cry1A.105 was assessed by SDS-PAGE analysis at three pH conditions; pH 10.3 (the protein's storage buffer), pH 1.2 and 7.5. The data show that the purified Cry1A.105 protein remains intact at these pH solutions.

[28] Dossier: Part II – Section 1.4.1.3a.
[29] Dossier: Part II – Section 1.4.1.3b.

The effect of pH on the intactness of the microbe-derived Cry2Ab2 was assessed by SDS-PAGE analysis at three pH conditions; pH 11.2 (the protein's storage buffer), pH 1.2 and 7.5. The data show that the purified Cry2Ab2 protein remains intact at these pH solutions.

3.4.3. Toxicology

3.4.3.1. Testing of the newly expressed proteins

The two proteins, Cry1A.105 and Cry2Ab2, newly expressed in soybean MON 87751 have been extensively characterised (Section 3.2.3).

The Cry1A.105 protein was previously assessed by the GMO Panel (e.g. EFSA, 2008)[30] and no safety concerns for humans and animals were identified. Updated bioinformatic analysis does not reveal similarities of the Cry1A.105 protein to known toxins causing toxicity to humans and animals (except for specific lepidopteran pests). The GMO Panel is not aware of any new information that would change the previous conclusion of the risk assessment that the Cry1A.105 protein does not raise safety concerns.

The Cry2Ab2 protein was previously assessed by the GMO Panel (e.g. EFSA, 2008)[31] and no safety concerns for humans and animals were identified. Updated bioinformatic analysis does not reveal similarities of the Cry2Ab2 protein to known toxins causing toxicity to humans and animals (except for specific lepidopteran pests). The GMO Panel is not aware of any new information that would change the previous conclusion of the risk assessment that the Cry2Ab2 protein does not raise safety concerns.

Based on scientific knowledge, no synergistic or antagonistic interactions between the two proteins newly expressed in soybean MON 87751 which could raise safety concerns for food and feed are expected.

The applicant provided studies on Cry1A.105 and Cry2Ab2 proteins from bacterial recombinant systems, which were considered equivalent to the plant-produced proteins (Section 3.2.3).

In vitro *degradation studies*[32]

The resistance of the microbe-derived Cry1A.105 and Cry2Ab2 proteins (*E. coli* produced MON 87751 Cry1A.105 and Cry2Ab2 proteins) to degradation by pepsin were investigated in solutions at pH ~ 1.2 in two independent studies. The integrity of the test proteins in samples of the incubation mixture taken at various time points was analysed by SDS-PAGE gel electrophoresis followed by protein staining or by Western blotting. No intact Cry1A.105 or Cry2Ab2 proteins were observed after 30 seconds of incubation. By protein staining analysis, a peptide fragment of 60 kDa was observed in samples of Cry1A.105 after 30 s of incubation but it was not seen at the 2 min incubation time point. In addition, low-molecular-weight fragments of ~ 4–5 kDa observed in samples of Cry1A.105 or Cry2Ab2 completely disappeared after 20 min or after 2 min of incubation, respectively. By western blot analysis, none of these fragments were immunologically reactive to a polyclonal antibody against either Cry1A.105 or Cry2Ab2.

Furthermore, the applicant performed a sequential digestion test based on a brief digestion (2 min) in a pepsin resistance test followed by digestion in so called simulated intestinal fluid (SIF) according to a method previously described (USP, 1995) in which SIF designates a mixture of proteolytic enzymes known as pancreatin. The purpose of such test was to better understand the digestive fate of Cry1A.105 protein and, particularly, to address the digestibility of the transiently stable ~ 5 kDa fragment visible in the early incubation time points of the pepsin degradation study. Under this scenario, the small fragment was completely digested within 30 seconds of SIF exposure.

In addition, the applicant also performed a standalone degradation study of Cry1A.105 and Cry2Ab2 proteins in SIF. The GMO Panel notes that the resistance to degradation by standalone SIF is currently not specifically required by either Regulation (EU) No 503/2013, EFSA guidance document (EFSA GMO Panel, 2011a) or Codex Alimentarius (2009). Due to the intrinsic limitations of such standalone SIF degradation study for the food and feed safety of newly expressed proteins, it is not considered in the overall safety assessment.

The main outcomes of the *in vitro* degradation studies detailed above are in line with those described for Cry1A.105 and Cry2Ab2 proteins previously assessed by the GMO Panel (EFSA, 2008).

[30] The amino acid sequence of Cry1A.105 in soybean MON 87751 differs from that previously assessed in maize MON 89034 (EFSA, 2008) by the presence of four additional amino acid residues at the N terminus. However, the remaining of the Cry1A.105 amino acid sequence in soybean MON 87751 is identical to that in maize MON 89034.

[31] The amino acid sequence of Cry2Ab2 in soybean MON 87751 differs from that previously assessed in maize MON 89034 (EFSA, 2008) by the lack of the first 18 amino acid residues at the N terminus. However, the remaining of the Cry2Ab2 amino acid sequence in soybean MON 87751 is identical to that in maize MON 89034.

[32] Dossier: Part II – Section 1.5.1.3.

Acute oral toxicity testing[33]

For compliance with Article 6 of Regulation (EU) No 503/2013, the applicant spontaneously submitted four acute toxicity studies in mice with Cry1a.105 and Cry2Ab2 proteins which were evaluated by the GMO Panel.

A microbe-derived Cry1a.105 protein was administrated in two separate studies at the doses of 2,000 mg/kg body weight (bw) or 5,000 mg/kg bw to male and female Crl:CD1(ICR) mice. No adverse effects related to the Cry1a.105 protein were observed.

A microbe-derived Cry2Ab2 protein was administrated in two separate studies at the dose of 2,000 mg/kg bw or 5,000 mg/kg bw to male and female Crl:CD1(ICR) mice. No adverse effects related to the Cry2Ab2 protein were observed.

28-day repeated dose toxicity study[34]

For compliance with Article 6 of Regulation (EU) No 503/2013, the applicant spontaneously submitted two 28-day repeated dose toxicity studies in mice with Cry1a.105 and Cry2Ab2 proteins which were evaluated by the GMO Panel.

Cry1a.105 protein

The applicant provided a 28-day oral repeated dose toxicity study in mice, conducted in accordance with OECD TG 407 (2008) and in compliance with the principles of Good Laboratory Practice (GLP).

Groups of singly caged Crl:CD1(ICR) mice (16 per sex per group, approximately seven weeks old at study start) were administered by gavage for 28 days the Cry1A.105 protein (in bicarbonate buffer solution) at targeted nominal doses of 10, 100 or 1,000 mg/kg bw per day (low-, mid- and high-dose Cry1A.105 protein groups) or bovine serum albumin (BSA) at a targeted nominal dose of 1000 mg/kg bw per day (BSA control group). No vehicle control group was included in the study.

The batch of the test substance contained 78% (wt/wt) of a recombinant Cry1A.105 protein produced in *E. coli*). Detailed information of the protein characterisation was provided with the certificate of analysis.[35] This protein has a molecular weight of 131.1 kDa.

Three dosing formulations of Cry1A.105 protein and BSA were prepared and provided as frozen aliquots for daily use. Samples of these formulations were used for concentration confirmation, homogeneity and stability analyses.

Feed and water were provided *ad libitum*. During the treatment period, the animals were checked twice daily for mortality and clinical signs. Detailed clinical observations were conducted on all animals before treatment and weekly during the treatment period. Ophthalmoscopy was performed before the start and during the last week of the treatment period. Individual body weights were recorded before treatment and weekly thereafter; body weight gains were calculated relative to the first day of treatment (day 0). Feed consumption was determined weekly. At the end of the treatment period, blood samples were taken for haematology and clinical chemistry analyses from up to ten animals (randomly selected) per sex and group; blood samples for coagulation analysis were taken from the remaining five or six animals per sex and group. All animals surviving the treatment period were sacrificed and underwent a detailed necropsy with determination of organ weights. Animals found dead or euthanised in extremis during the treatment period also underwent a detailed necropsy examination. Organs and tissues from those animals assigned to haematology and clinical chemistry analyses in the high-dose Cry1A.105 protein group, the BSA control group and those found dead or euthanized in extremis were subjected to a comprehensive histopathological examination. Histopathology of gross lesions was conducted throughout groups. A pathology peer review was conducted.

For all continuous endpoints mean, standard deviation and the number of animals were reported for each treatment group. The original statistical analysis provided was based on one-way ANOVA model followed, in case of statistically significant ($p < 0.05$) intergroup variance, by a Dunnett's test to compare the Cry1A.105-treated groups to the BSA-treated group. Due to the study design including a high-dose (1,000 mg/kg bw per day) BSA administration as the only control, the GMO Panel asked for a new statistical analysis assessing effects in the 1,000 mg/kg bw per day Cry1A.105 protein group as compared to the 1,000 mg/kg bw per day BSA group only. In response to this EFSA request[36], body weight, body weight change, feed consumption, clinical pathology and organ weight data were

[33] Additional information: 20/12/2016.
[34] Additional information: 5/10/2017.
[35] The purity of the test substance was taken into account in the formulation.
[36] Additional information received on 3/5/2018.

analysed, by sex, using two-sample t-test at 5% significance level to compare high-dose test group (1,000 mg/kg bw per day, Group 4) to the BSA-treated group. Histopathological findings data were analysed using one-sided Fisher's exact test at 5% significance level.

The Cry1A.105 and BSA proteins concentration, homogeneity and stability were confirmed.

No test substance-related mortality was observed. One male of the BSA control group was found dead on day 13 of the study; the cause of death was not established. Three animals given the Cry1A.105 protein at a dose of 1000 mg/kg bw per day were found dead or were sacrificed for humane reasons (one male on day 3 and two females on day 14 and 17) as a consequence of gavage errors.

No test diet-related clinical signs and ophthalmoscopic findings were observed.

A statistically significant increase in mean final body weight (day 28) and cumulative body weight gain (days 0–7 and 0–28) were observed in females given the Cry1A.105 protein, compared to the BSA control group (~ 6%). Due to the low magnitude of the increase in the final body weight, the GMO Panel does not consider these changes as adverse.

Haematological analysis showed a statistically significant decrease in the red blood cell count (~ 9%), haemoglobin concentration (~ 7%) and haematocrit (~ 8%), and an increase in the red blood cell distribution width (~ 8%) in males given the Cry1A.105 protein, compared to the BSA control group. These differences were small, not associated with test substance-related changes in related parameters (e.g. reticulocyte count, gross pathology and histopathology of spleen and bone marrow) and therefore, the GMO Panel does not consider these effects as being adverse.

A statistically significant increase in mean absolute neutrophil count (~ 47%) was observed in males given the Cry1A.105 protein, compared to the BSA control group. Although substantial, this difference was not associated with test substance-related changes in related parameters (e.g. percentage of neutrophils, white blood cell count, histopathology of thymus and bone marrow). Therefore, the GMO Panel does not consider this isolated finding as an adverse effect.

A statistically significant increase in mean absolute LUC[37] count (~ 100%) was observed in males given the Cry1A.105, compared to the BSA control group. Although substantial, this increase was not associated with an increase in the percentage of LUC or changes in related endpoints of the white blood cell population (e.g. lymphocytes, mononuclear cells). Moreover, no histopathological findings potentially related to increased LUC count was reported (e.g. histopathology of reticuloendothelial, haematopoietic and lymphoid tissues). Therefore, the GMO Panel does not consider this isolated finding as an adverse effect.

Clinical chemistry analysis showed a statistically significant decrease in mean albumin (~ 6%), albumin/globulin (A/G) ratio (~ 5%), sodium (~ 1.7%) and chloride (~ 1.3%) concentrations in males given the Cry1A.105 protein, compared to the BSA control group. These differences were minimal and therefore, the GMO Panel does not consider these changes to be adverse.

Organ weight determinations showed a statistically significant increase in mean spleen weight (absolute, 28%; relative to body weight, 27%) and relative liver weight (~ 6%) in males given the Cry1A.105 protein, compared to the BSA control group. No test substance-related histologic changes were noted in these organs and therefore, the GMO Panel does not consider these changes as adverse effects.

A statistically significant decrease in mean adrenal gland weights (absolute, 12%; relative to body weight, 17%; and relative to brain weight, 8%) was observed in females given the Cry1A.105 protein, compared to the BSA control group. No test substance-related histologic changes were noted in these organs and therefore, the GMO Panel does not consider these changes as adverse effects.

Macroscopic examinations performed at necropsy on all animals revealed no gross pathological findings related to the administration of the Cry1A.105 protein. Microscopic examinations of selected organs and tissues identified no test substance-related differences in the incidences and severity of the histopathological findings between the groups.

The GMO Panel notes the following deviations from OECD TG 407 requirements: coagulation examinations were based on a relatively low number of evaluable samples (in most of the cases below the minimum requirement of five animals/gender per group). Functional observational battery (FOB) and locomotor activity tests were not performed.

Regarding the coagulation analysis, the GMO Panel considered that the integrated assessment of related parameters (e.g. no difference in the platelet count or in the spleen and bone marrow histopathology between groups) indicates that an effect on coagulation parameters is unlikely. The

[37] Large unstained cells, i.e. large peroxidase-negative cells that cannot be characterised further as large lymphocytes, 'virocytes' or stem cells.

GMO Panel therefore concludes that this is not a major deviation compromising the safety assessment of the Cry1A.105 protein.

Regarding the lack of FOB and locomotor activity tests (tests performed to identify potential neurotoxicity of a test substance), the GMO Panel used a weight of evidence approach to exclude potential neurotoxic effects of the Cry1A.105 protein: (1) in this 28-day study, no test substance-related findings were noted in the daily clinical examinations or in the detailed clinical examinations (performed approximately weekly, including removal from the home cage); (2) no test substance-related effects were seen in the acute oral toxicity studies in mice given 2,000 or 5,000 mg Cry1A.105 protein/kg bw; (3) no similarity of the Cry1A.105 protein to known neurotoxicants was identified in bioinformatic analysis; (4) according to current knowledge, there is no indication of neurotoxicity for Cry proteins (e.g. EFSA GMO Panel, 2016a–c, 2017a,b). The GMO Panel therefore considers that the lack of FOB and locomotor activity tests is not a major deviation compromising the safety assessment of the Cry1A.105 protein.

The GMO Panel concludes that no adverse effects are observed in this mouse 28-day toxicity study on Cry1A.105 protein at the highest dose tested (1,000 mg/kg bw per day).

Cry2Ab2 protein

The applicant provided a 28-day oral repeated dose toxicity study in mice, conducted in accordance with OECD TG 407 (2008) and in compliance with the GLP principles.

Groups of singly caged Crl:CD1(ICR) mice (16/sex per group, approximately seven weeks old at study start) were administered by gavage for 28 days the Cry2Ab2 protein (in carbonate-bicarbonate buffer solution) at targeted nominal doses of 10, 100, or 1,000 mg/kg bw per day (low-, mid- and high-dose Cry2Ab2 protein groups) or BSA in the same vehicle at a targeted nominal dose of 1,000 mg/kg bw per day (BSA control group). No vehicle control group was included in the study.

The two batches of the test substance contained 71% and 76% (wt/wt) of a recombinant Cry2Ab2 protein produced in *E. coli*. Detailed information on the protein characterisation was provided with the certificate of analysis.[34] This protein has a molecular weight of 59.5 kDa.

Three dosing formulations of Cry2Ab2 protein and BSA were prepared and provided as frozen aliquots for daily use. Samples of these formulations were used for concentration confirmation, homogeneity and stability analyses.

Feed and water were provided *ad libitum*. During the treatment period, the animals were checked twice daily for mortality and clinical signs. Detailed clinical observations were conducted on all animals before treatment and weekly during the treatment period. Ophthalmoscopy was performed before the start and during the last week of the treatment period. Individual body weights were recorded before treatment and weekly thereafter; body weight gains were calculated relative to the first day of treatment (day 0). Feed consumption was determined weekly. At the end of the treatment period, blood samples were taken for haematological and clinical chemistry analyses from up to ten animals (randomly selected) per sex and group; blood samples for coagulation analysis were taken from the remaining six animals per sex and group. All animals surviving the treatment period were sacrificed and underwent a detailed necropsy with determination of organ weights. Animals found dead during the treatment period also underwent a detailed necropsy examination. Organs and tissues from those animals assigned to haematological and clinical chemistry analyses in the high-dose Cry2Ab2 protein group, the BSA control group and those found dead were subjected to a comprehensive histopathological examination. Histopathology of gross lesions was conducted throughout groups. A pathology peer review was conducted.

For all continuous endpoints mean, standard deviation and the number of animals were reported for each treatment group. The original statistical analysis provided was based on one-way analysis of variance (ANOVA) model followed, in case of statistically significant ($p < 0.05$) intergroup variance, by a Dunnett's test to compare the Cry2Ab2-treated groups to the BSA-treated group. Due to the study design including a high-dose (1,000 mg/kg bw per day) BSA administration as the only control, the GMO Panel asked for a new statistical analysis assessing effects in the 1,000 mg/kg bw per day Cry2Ab2 protein group as compared to the 1,000 mg/kg bw per day BSA group only. In response to this EFSA request,[35] body weight, body weight change, feed consumption, clinical pathology and organ weight data were analysed, by sex, using two-sample t-test at 5% significance level to compare high-dose test group (1,000 mg/kg bw per day, Group 4) to the BSA-treated group. Histopathological findings data were analysed using one-sided Fisher's exact test at 5% significance level.

The Cry2Ab2 and BSA protein concentration, homogeneity and stability were confirmed.

No test substance-related mortality was observed. One male of the BSA control group and one female given the test substance at 100 mg/kg bw per day were found dead during the study, as a consequence of gavage errors.

No test diet-related clinical signs and ophthalmoscopic findings were observed.

A statistically significant decrease in mean feed consumption (g/animal/day, ~ 16%) was observed in males given the Cry2Ab2 protein, compared to the BSA control group, during study days 7 to 14. This decrease was not associated with differences in the mean final and mean cumulative body weights and thus not toxicologically relevant.

Haematological analysis showed a statistically significant increase in mean absolute neutrophil count (~ 150%) in females given the Cry2Ab2 protein, compared to the BSA control group. Although the difference was substantial, it was not associated with test substance-related changes in related parameters (e.g. percentage of neutrophils, white blood cell count, histopathology of thymus and bone marrow). Therefore, the GMO Panel does not consider this isolated finding as an adverse effect.

A statistically significant decrease in mean corpuscular volume (MCV) (~ 3.6%) and increase in mean corpuscular haemoglobin concentration (~ 3%) were also observed in females given the Cry2Ab2 protein, compared to the BSA control group. The differences in these calculated parameters were minimal and not associated with test substance-related changes in related parameters (e.g. red blood cell count, haemoglobin concentration, haematocrit), and is therefore not considered as adverse effects by the GMO Panel.

Clinical chemistry analysis showed a statistically significant decrease in mean triglyceride levels (~ 23%) in males given the Cry2Ab2 protein, compared to the BSA control group. The GMO Panel considers this small change as not being adverse. A statistically significant decrease in mean A/G ratio (~ 8%) was observed in females given the Cry2Ab2 protein, compared to the BSA control group. The difference was small and therefore, the GMO Panel does not consider this change as an adverse effect.

Organ weight determinations showed a statistically significant increase in mean weights (relative to final body weights only) of brain (~ 4%), epididymides (~ 10%) and spleen (~ 17%) in males given the Cry2Ab2 protein, compared to the BSA control group. No test substance-related histological changes were noted in these organs, and therefore, the GMO Panel does not consider these changes as adverse effects.

Macroscopic examinations performed at necropsy on all animals revealed no gross pathological findings related to the administration of the Cry2Ab2 protein. Microscopic examinations of selected organs and tissues identified no test substance-related differences in the incidences and severity of the histopathological findings between the groups.

The GMO Panel noted the following deviations from OECD TG 407 requirements: coagulation examinations were performed on six animals per gender per group, except for high-dose females (four animals examined). FOB and locomotor activity tests were not performed.

Regarding the coagulation analysis, the GMO Panel considered that the integrated assessment of related parameters (e.g. no difference in the platelet count or in the spleen and bone marrow histopathology between groups) indicates that an effect on coagulation parameters is unlikely. The GMO Panel therefore concludes that this is not a major deviation compromising the safety assessment of the Cry2Ab2 protein.

Regarding the lack of FOB and locomotor activity tests (tests performed to identify potential neurotoxicity of a test substance) the GMO Panel used a weight of evidence approach to exclude potential neurotoxic effects of the Cry2Ab2 protein: (1) in this 28-day study no test substance-related findings were noted in the daily clinical examinations or in the detailed clinical examinations (performed approximately weekly, including removal from the home cage); (2) no test substance-related effects were seen in the acute oral toxicity studies in mice given 2,000 or 5,000 mg Cry2Ab2 protein/kg bw; (3) no similarity of the Cry2Ab2 protein to known neurotoxicants was identified in bioinformatic analysis; (4) according to current knowledge, there is no indication of neurotoxicity for Cry proteins (e.g. EFSA GMO Panel, 2016a–c, 2017a,b). The GMO Panel therefore considers that the lack of FOB and locomotor activity tests is not a major deviation compromising the safety assessment of the Cry2Ab2 protein.

The GMO Panel concluded that no adverse effects is observed in this mouse 28-day toxicity study on Cry2Ab2 protein at the highest dose tested (1,000 mg/kg bw per day).

3.4.3.2. Testing of new constituents other than newly expressed proteins

No new constituents other than newly expressed proteins have been identified in soybean MON 87751. Therefore, no further food and feed safety assessment of components other than the newly expressed proteins is required.

3.4.3.3. Information on altered levels of food/feed constituents

None of the differences identified in seed and forage composition between soybean MON 87751 and its conventional counterpart (Section 3.3.4) require further assessment.

3.4.3.4. Testing of the whole genetically modified food and feed

No substantial modifications in the composition of soybean MON 87751, no indication of possible unintended effects relevant for food/feed safety were identified. Therefore, animal studies on the food/feed derived from soybean MON 87751 are not necessary (EFSA GMO Panel, 2011a). In accordance with Regulation (EU) No 503/2013 the applicant provided a 90-day oral repeated-dose toxicity study on whole food and feed from soybean MON 87751 in rats. Animal feeding studies in broilers and channel catfish fed diets containing soybean MON 87751 were also provided in compliance with Article 6 of Regulation (EU) No 503/2013. All these studies are evaluated by the GMO Panel.

90-day feeding study in rats[38]

Pair-housed Crl:CD(SD) rats (16/sex per group) were allocated to two groups using a randomised complete block design with eight replications. Groups were fed test or control diets containing approximately 30% (w/w) toasted defatted meal from soybean MON 87751 (test item) or from the conventional counterpart (control material), respectively. The study provided was adapted from OECD TG 408 and complying with the GLP principles.

Event-specific PCR analysis on seeds[39] prior to processing into meal confirmed the molecular identity of soybean MON 87751 seeds and the lack of soybean MON 87751 contamination in the conventional counterpart. Both test item and control materials were analysed for proximates, amino acids, minerals, antinutrients and pesticides.

Balanced diets[40] were prepared according to the specifications for PMI Certified Rodent LabDiet #5002, with a soybean meal inclusion rate of 30% (w/w).

Stability of the test and control materials was not tested; however in accordance to product expiration standards declared by the diet manufacturer, the constituents of the diets are considered stable for the duration of the treatment. There are currently no practical analytical methods available to determine homogeneity, and concentration of toasted defatted soybean meal in the formulated diets. Diet preparation procedures and regular evaluations of the mixing methods by surrogate analytes guaranteed their homogeneity and the proper concentration of the test or control material in them.

Feed and water were provided *ad libitum*.

Animals were checked twice daily for mortality and clinical signs. Detailed physical examinations were conducted on all animals pre-treatment and then weekly during the dosing period, and on the day of the scheduled necropsy. Individual body weights were recorded pretreatment and then weekly during the dosing period and on the day prior to the scheduled necropsy. Feed consumption (per cage) was determined weekly during the study.

Ophthalmoscopy and FOB data and locomotor activity were recorded on all animals pre-treatment and at the end of the study (week 12). Clinical pathology (i.e. haematology, clinical chemistry and coagulation, urine analyses) and necropsy examination with organs weighing were conducted at the end of the treatment period on all animals. The animals were fasted overnight prior to blood collection while in metabolism cages for urine collection. Organs and tissues from all sacrificed animals as well as gross lesions were subjected to a detailed histopathological examination. Upon completion of the histopathologic assessment of all tissues, histopathology was reviewed by a peer review pathologist.

Mean, median, standard deviation, min and max were reported for all continuous endpoints for each group/sex and per period or time as appropriate. The applicant performed a power analysis per gender, using a prespecified effect sizes[41] for eight endpoints[42] with a 5% level of significance. For all selected endpoints the power estimates were greater than 99%, with the exception of absolute lymphocytes (80% for males and 60% for females), and body weight (80% for males and 88% for females). The cage or the individual animal was considered the experimental unit according to the corresponding estimate of

[38] Dossier: Part II – Section 1.4.4.1
[39] Additional information: 20/4/2016.
[40] Additional information: 20/4/2016 and 21/7/2016.
[41] Defined on the basis of six previous studies: WIL-50283 (Kirkpatrick, 2005), WIL-50296 (Kirkpatrick, 2007), WIL-50297 (Kirkpatrick, 2007), WIL-50333 (WIL-WIL-50370 (Kirkpatrick, 2010) and additional information 22/9/2016.
[42] Eight endpoints were selected: absolute lymphocytes, alkaline phosphatase, body weight, cholesterol, creatinine, urea nitrogen, kidney weight, liver weight.

cage effect. The in-life and terminal body weights/gain, organ weights, feed consumption/efficiency and clinical pathology and functional observations when appropriate, parameters were checked for homogeneity and normality,[43] analysed with ANOVA and tested using a t-tests. Finally, outcome proportions of incidence of functional observations were analysed with Fisher's exact test. In response to a request from the GMO Panel[44] the difference between test and control groups and associated 95% confidence interval were also presented in terms of standardised effect size (i.e. normalised to standard deviation[45]). The goodness-of-fit was evaluated by visual examination of residual plots and histograms.[46] Based on this evaluation the models were considered appropriate.

All animals survived the treatment period. No test diet related clinical signs and ophthalmoscopic findings were observed.

No statistically significant differences in mean body weights and in mean body weight gains are observed between animals fed the test and control diets. No treatment-related differences in feed consumption are noted.

No statistically significant differences in FOB and locomotor activity parameters are observed between animals fed the test and control diets at the end of the study, with the exception of tail pinch response and hindlimb footsplay, in males fed the test diet, compared to control. These were the only changes among the parameters examined and therefore not considered to be test substance related.

Statistically significant lower mean absolute neutrophil count and mean absolute monocyte count were observed, respectively, in males and in females fed the test diet, as compared to the corresponding control group. The GMO Panel considers these isolated findings not to be treatment-related.

Statistically significant lower mean red blood cell count, haemoglobin concentration, reticulocyte number (absolute reticulocytes), and higher MCV were observed in females fed the test diet compared to control. These findings were not associated with changes in haematopoietic tissue histopathology (e.g. sternum bone marrow and spleen) and therefore are not considered toxicologically relevant.

Statistically significant decreases in mean albumin (2.5%) and total protein (3%) were observed in males fed the test diet. These minimal differences are not considered to be toxicologically relevant.

Lower mean alkaline phosphatase values were observed in females fed the test diet compared the respective controls. This finding was not associated with changes in related endpoints (e.g. liver histopathology) and is not considered toxicologically relevant.

Statistically significant higher urine mean specific gravity and a slightly lower mean pH value were observed in females fed test diet, compared to controls. In the absence of changes related to renal pathology, these findings are not considered toxicologically relevant.

No statistically significant differences in organ weight were observed among male and female rats fed test diets and controls, except for a lower mean absolute brain weight (3.2%) in males and a lower mean relative liver weight (3%) in females fed the test diets, compared to controls. These changes are not considered to be adverse because of their magnitude and because the decrease in the relative liver weight is not associated with changes in related endpoints (e.g. liver histopathology).

No treatment-related gross lesions or microscopic findings were noted in organs or tissues. Sporadic histopathological findings are considered compatible with the spontaneous background pathology of rats of this strain and age.

The GMO Panel concludes that no soybean MON 87751-related adverse effects were observed in this study.

The GMO Panel notes that the applicant tested only one dose level. However, the dose tested was close to the highest possible without inducing nutritional imbalance according to the current knowledge, and in accordance to the limit test dose as described in OECD TG 408. Therefore, this is not considered to affect the above conclusions.

A second 90-day feeding study in rats on defatted toasted meal from soybean MON 87551 was submitted. The GMO Panel notes that balanced diets were prepared including test and control materials stored for more than one year, without any check for stability.[47] For this reason, the GMO Panel does not further consider this study in the assessment.

[43] Normality and heterogeneity assumptions were checked by visual examination of residual plots and histogram. No extreme violations of the assumptions were observed.
[44] Additional information received on 22/9/2016 and 20/12/2016.
[45] Additional information 20/12/2016.
[46] Additional information 11/4/2017.
[47] Additional information received: 31/5/2017.

42-day broiler study[48]

A total of 800 (400 per sex) one-day old chicken broilers (Cobb × Cobb 500) were randomly allocated to eight dietary groups with 100 chicks per treatment (ten pens per treatment, half for each sex, ten birds per pen) and fed balanced diets[49] containing up to 35%[50] soybean meal from soybean MON 87751 (test diet), the conventional counterpart A3555 (control diet) or one of the six reference varieties Midwest Genetics G2712, Stewart SB3454, Hoffman H387, Williams 82, Gateway 505, Horizon Genetics H5310N (reference diets). Diets (as crumbled pellets or pellets) and water were offered *ad libitum*. No statistically significant differences between the groups fed test and control diets were observed in the majority of growth performance and carcass parameters: mortality (2.5%), final body weight, weight gain, feed to gain ratio, and yield of pre-chill organs and post-chilled carcass and cuttable parts (absolute and relative weights). A higher breast weight and lower thigh weight percentages were observed in the test group, compared to the control group; these findings, since not associated to changes in the respective absolute weights and in carcass yield, were not considered adverse. Some statistically significant differences (i.e. adjusted feed to gain ratio and breast meat, thigh and drum weight percentages) were observed between the test group and one or two out of six reference groups. Because the differences were not observed between the test group and the majority of the reference groups, they are not considered relevant.

The GMO Panel concludes that administration of diets containing up to 35% soybean meal MON 87751 to broilers does not cause adverse effects and that the measured performance endpoints were similar between groups fed balanced diets containing GM and non-GM soybean (conventional counterpart and references).

8-week channel fish study[51]

A total of 600 channel catfish (sex undetermined) were randomly allocated to six dietary groups with 100 catfishes per treatment (five aquaria per treatment, twenty fishes per aquaria) and fed balanced diets formulated as sinking pellets containing approximately 45% soybean meal from soybean MON 87751 (test diet), the conventional counterpart A3555 (control diet) or one of the four reference varieties Midwest Genetics G2712, Stewart SB3454, Hoffman H387, Gateway 505 (reference diets). No mortality and no abnormal behaviours were observed among fishes in any aquarium during the study. There were no statistically significant differences in overall weight gain per fish, total diet consumption per fish, or diet conversion ratio among fish fed the test, control and reference diets.

The GMO Panel concludes that administration of balanced diets containing up to 45% soybean meal MON 87751 to channel catfish does not cause adverse effects and that the measured performance endpoints were similar between groups fed balanced diets containing GM and non-GM soybean (conventional counterpart and references).

3.4.4. Allergenicity

The strategies to assess the potential risk of allergenicity focus on the source of the recombinant protein, on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and on whether the transformation may have altered the allergenic properties of the modified plant.

3.4.4.1. Assessment of allergenicity of the newly expressed proteins[52]

A weight-of-evidence approach was followed, taking into account all the information obtained on the newly expressed protein, as no single piece of information or experimental method yield sufficient evidence to predict allergenicity (Codex Alimentarius 2009; EFSA GMO Panel, 2011a; Regulation (EU) No 503/2013).

The *cry1A.105* and *cry2Ab2* genes originate from *B. thuringiensis*, which is not considered to be an allergenic source.

Updated bioinformatic analyses[53] of the amino acid sequences of the Cry1A.105 and Cry2Ab2 proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no

[48] Additional information received: 8/2/2016.
[49] Starter (0–21 days) and grower/finisher (22–42 days) diets.
[50] ~ 35% in starter diets; ~ 31% in grower/finisher diets.
[51] Additional information received 8/2/2016.
[52] Dossier: Part II – Section A1.5.1, A1.3.4.2 and additional information: 20/12/2016 and 15/5/2017.
[53] Additional information: 19/5/2017.

significant similarities to known allergens. In addition, the applicant performed analyses searching for matches of eight contiguous identical amino acid sequences between the Cry1A.105 and Cry2Ab2 proteins and known allergens, which confirmed the outcome of the previous bioinformatic analyses.

The studies on resistance of the Cry1A.105 and Cry2Ab2 proteins to degradation by pepsin have been described in Section 3.4.3.1.

The GMO Panel has previously evaluated the safety of the Cry1A.105 and Cry2Ab2 proteins in maize MON 89034 and no concerns on allergenicity were identified (EFSA, 2008).

For adjuvanticity, proteins derived from *B. thuringiensis* (*Bt* proteins) have been suggested to possess adjuvant activity based on laboratory animal studies on Cry1Ac when applied at relatively high doses (e.g. Vazquez et al., 1999). The GMO Panel has previously evaluated the safety of the Cry1A.105 and Cry2Ab2 proteins and no concerns on adjuvanticity in the context of the applications assessed were identified (EFSA, 2008). From the limited knowledge and experimental evidence available, the Panel does not find indications that the presence of the *Bt* proteins at the levels expressed in seeds from soybean MON 87751 might act as adjuvants with the potential to enhance a specific immunoglobulin E (IgE) response and to favour the development of an allergic reaction. With regard to forage, in which the levels of the Cry1A.105 and Cry2Ab2 proteins were higher than in seeds, the GMO Panel has no indications that these Cry proteins may have an adjuvant effect in animals fed forage.

In the context of the present application, the GMO Panel considers that there are no indications that the newly expressed Cry1A.105 and/or Cry2Ab2 proteins in soybean MON 87751 may be allergenic.

3.4.4.2. Assessment of allergenicity of the whole GM plant or crop[54]

Soybean is considered to be a common allergenic food[55] (OECD, 2012). Therefore, any potential change in the endogenous allergenicity of the GM plant should be assessed (Regulation (EU) No 503/2013). For such assessment, the applicant included in the comparative analysis specific allergens relevant for soybean (Section 3.3.4) that were measured by specific ELISA methods, which have been previously considered as acceptable (EFSA GMO Panel, 2010c; Fernandez et al., 2013; Selb et al., 2017). The applicant also referred to the Kunitz trypsin inhibitor as a potential soybean allergen, which is an antinutrient and as such it is also an endpoint already assessed in the compositional analysis (see Section 3.3.4). These allergens were selected based on the list of potential soybean allergens described in the pertinent OECD document (OECD, 2012) and a scientific rational supporting such selection was provided by the applicant and considered acceptable by the GMO Panel. No changes in the levels of endogenous allergens raising concern are identified by the GMO Panel.

Furthermore, the applicant provided two additional studies not requested by the GMO Panel. First, an investigation of the endogenous allergenicity comparing whole protein extracts of soybean MON 87751 and its conventional counterpart by gel electrophoresis analysis was conducted. The identity of bands/spots corresponding to specific soybean allergens were confirmed by mass spectrometry and their intensity were assessed by image analysis. No relevant changes in the intensity of the bands/spots between the protein extracts of soybean MON 87751 and its conventional counterpart were identified. Second, the IgE-binding capacity of whole protein seed extracts from soybean MON 87751, its conventional counterpart and different reference soybean varieties was also investigated using sera from four individuals allergic to soybean by EAST (enzyme allergosorbent test). Whole proteins in extracts from soybean MON 87751, the conventional counterpart and the reference varieties used had similar reactivity to the sera from the four allergic individuals.

In the context of this application, the GMO Panel considers that there is no evidence that the genetic modification might significantly change the overall allergenicity of soybean MON 87751 when compared with that of the conventional counterpart and the non-GM commercial reference soybean varieties tested.

[54] Dossier: Part II – Section 1.5.2 and additional information: 20/12/2016 and 15/5/2017.

[55] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

3.4.5. Dietary exposure assessment to endogenous and new constituents

In line with Regulation (EU) No 503/2013, the applicant provided dietary exposure to Cry1A.105 and Cry2Ab2 proteins produced in soybean MON 87751.

3.4.5.1. Human dietary exposure[56]

Only acute dietary exposure estimates to Cry1A.105 and Cry2Ab2 proteins were provided by the applicant. Dietary exposure was estimated across different European countries on different population groups: young population (toddlers, other children) and adult population (adolescents, adults, elderly and very elderly).

For the purpose of estimating dietary exposure, the levels of Cry1A.105 and Cry2Ab2 proteins in soybean MON 87751 seeds were derived from the 2012 USA field trial study (see Table 1 in Section 3.2.4); mean values of 2.1 μg/g and 3.6 μg/g (fresh weight) were used for Cry1A.105 and Cry2Ab2, respectively. Since no specific consumption data were available on consumption of commodities containing MON 87751 soybean, a conservative scenario with 100% replacement of conventional soybean by the GM soybean was considered. Consumption figures for the relevant commodities were retrieved by the applicant from the available summary statistics of the EFSA Comprehensive European Food Consumption Database.[57] The EFSA consumption database contains information on food consumption data at individual level from the most recent national dietary surveys in different EU Member States (EFSA, 2011; Huybrechts et al., 2011; Merten et al., 2011). Soybean oil was excluded from the assessment since no proteins are expected to be present in the oil.

For acute exposure estimations, the applicant used the protein content in the different soybean derived commodities to estimate the concentration of Cry1A.105 and Cry2Ab2 proteins in the consumed foods. This is considered a conservative approach as no losses of Cry1A.105 and Cry2Ab2 are assumed during processing. For each population group, the food commodity among consumers only (95th or 97.5th percentile depending on the number of consumers; EFSA 2011) leading to the highest acute exposure was selected. In 'Other children' (3–10 years), the highest acute exposure was estimated as 6.2 μg/kg bw day and 10.6 μg/kg bw day for Cry1A.105 and Cry2Ab2, respectively, following the consumption of soya drink. In adults (18–65 years), the highest acute exposure was estimated as 17.9 μg/kg bw day and 30.7 μg/kg bw day for Cry1A.105 and Cry2Ab2, respectively, following the consumption of meat imitates (textured soy protein). The use of the highest acute consumption for only one food commodity could slightly underestimate the dietary exposure to Cry1A.105 and Cry2Ab2 proteins in certain population groups.

The GMO Panel estimated chronic dietary exposure to Cry1A.105 and Cry2Ab2 proteins, since this was not provided by the applicant. Individual consumption data of the relevant food commodities were retrieved from the EFSA Comprehensive European Food Consumption Database, using dietary surveys with at least two days consumption and covering a total of 19 European countries.[58] Different recipes and processing factors were considered to estimate the amount of soybean in the consumed commodities before assigning Cry1A.105 and Cry2Ab2 levels to the relevant commodities.[59] No losses in Cry1A.105 and Cry2Ab2 during processing were considered. For protein Cry1A.105, the highest mean chronic dietary exposure in the young population was estimated in toddlers (1–3 years) with 0.36 μg/kg bw day, while the highest 95th percentile exposure was in 'Other children' (3–10 years) with 0.60 μg/kg bw day. Corresponding highest estimates in adults were 0.05 μg/kg bw day and 0.28 μg/kg bw day for mean and 95th percentile exposure, respectively. Similarly, for protein Cry2Ab2, the highest chronic dietary exposure in the young population was estimated in toddlers (0.61 μg/kg bw day), and the highest 95th percentile exposure (1.02 μg/kg bw day) in 'Other children'. Corresponding highest estimates in adults were 0.09 μg/kg bw day and 0.49 μg/kg bw day for mean and 95th percentile exposure, respectively. Overall, in those dietary surveys with the highest chronic exposure the main contributor to the exposure was soymilk. A conservative exposure scenario to cover possible daily consumption of protein isolates derived from soybean MON 87751 as protein supplements was carried out. Considering a total protein concentration of around 90% in these isolates, the concentration of Cry1A.105 and Cry2Ab2 would be approximately 5 μg/g and 9 μg/g, respectively. Based on the EFSA NDA Panel scientific opinion on dietary

[56] Dossier: Part II – Section 2.4.
[57] https://www.efsa.europa.eu/en/applications/gmo/tools
[58] Austria, Belgium, Bulgaria, Cyprus, the Czech Republic, Germany, Denmark, Spain, Finland, France, United Kingdom, Greece, Hungary, Ireland, Italy, Latvia, the Netherlands, Romania, and Sweden.
[59] Example: 100 g of tofu is made with 20.4 g of soybeans; this would result in 0.43 μg of Cry1A.105 per gram of tofu as compared to 2.1 μg/g in the soybeans.

reference values for protein (EFSA NDA Panel, 2012), a high consumer of protein (95th percentile) in the European adult population can consume as much as 1.90 g/kg bw day while in children would reach 4.73 g/kg bw day. Assuming that all consumed protein was from soybean MON 87751 soybean isolates, the estimated exposure to Cry1A.105 and Cry2Ab2 in adults would be around 11 μg/kg bw day and 19 μg/kg bw day, respectively. In children, the estimated exposure would be around 28 μg/kg bw day and 47 μg/kg bw day to Cry1A.105 and Cry2Ab2, respectively.

3.4.5.2. Animal dietary exposure[60]

Daily dietary exposure (DDE) to Cry1A.105 and Cry2Ab2 proteins in soybean MON87751 was provided by the applicant across different animal species (i.e. broiler, finishing pig and lactating dairy cattle), based on estimates, as provided for the EU by OECD (OECD, 2009), for animal body weight, daily feed intake and inclusion rates (percentage) of soybean meal in animal diets. A conservative scenario with 100% replacement of conventional soybean (soybean meal) by the GM soybean was considered. The mean levels of Cry1A.105 and Cry2Ab2 proteins in seeds derived from the field trial study performed in 2012 (see Table 1 in Section 3.2.4) were used as reference to estimate the mean protein levels in soybean meal,[61] calculated to be 1.28-fold higher than in soybean seed, based on the protein content of soybean meal relative to soybean seed (OECD, 2012), assuming that no protein is lost during the processing.

Estimated DDEs to the Cry1A.105 based on the consumption of GM soybean meal was 87 μg/kg bw in broiler, 30 μg/kg bw in dairy cattle and 28 μg/kg bw in finishing pig.

Estimated DDEs to the Cry2Ab2 based on the consumption of GM soybean meal was 145 μg/kg per bw in broiler chickens, 49 μg/kg per bw in dairy cattle, and 46 μg/kg per bw in finishing pig.

The GMO Panel estimated DDEs to Cry1A.105 and Cry2Ab2 proteins in lactating dairy cows, based on estimates for animal body weight and daily feed intake, as provided for the EU by OECD (OECD, 2009), and estimates for inclusion rates of soybean forage/silage in animal diets, as provided by OECD consensus document (OECD, 2012) (information that was not provided by the applicant). A conservative scenario with 100% replacement of conventional soybean (forage/silage) by the GM soybean was considered. Mean levels of Cry1A.105 and Cry2Ab2 proteins in forage derived from the field trials performed in 2012 (see Table 1 in Section 3.2.4) were used as occurrence data. Estimated DDEs in lactating dairy cows, based on the consumption of GM soybean forage/silage was 1771 μg/kg per bw for Cry1A.105 and 108 μg/kg per bw for Cry2Ab2.

3.4.6. Nutritional assessment of GM food and feed[62]

The intended trait of soybean MON 87751 is insecticide resistance, with no intention to alter the nutritional parameters. Comparison of the seed and forage composition of soybean MON 87751 with the conventional counterpart and the non-GM commercial reference varieties did not identify differences that would require a nutritional assessment as regards food and feed (see Section 3.3.4). From these data, the GMO Panel concludes that the nutritional impact of soybean MON 87751-derived food and feed is expected to be the same as that from its conventional counterpart and non-GM commercial reference varieties.

3.4.7. Post-market monitoring of GM food and feed[63]

The GMO Panel concludes that soybean MON 87751, as described in this application, is nutritionally equivalent to and as safe as the conventional counterpart and the non-GM soybean reference varieties tested, and no post-market monitoring (EFSA GMO Panel, 2011a) of food/feed is considered necessary.

3.4.8. Conclusion on food and feed safety assessment

The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the proteins Cry1A.105 and Cry2Ab2 expressed in soybean MON 87751, and finds no evidence that the genetic modification might significantly change the overall allergenicity of soybean MON 87751. Based on the outcome of the comparative assessment, the nutritional impact of soybean MON 87751-derived food and feed is expected to be the same as those derived from its conventional counterpart and

[60] Dossier: Part II – Section 2.3.
[61] Cry1A.105: 230 μg/g; Cry2Ab2: 14 μg/g.
[62] Dossier: Part II – Section 1.6.
[63] Dossier: Part II – Section 4.

non-GM commercial reference varieties. The GMO Panel concludes that soybean MON 87751 is nutritionally equivalent to and as safe as the conventional counterpart and the non-GM reference varieties tested.

3.5. Environmental risk assessment and monitoring plan

Considering the scope of application EFSA-GMO-NL-2014-121, which excludes cultivation, the ERA of soybean MON 87751 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable soybean MON 87751 seeds during transportation and processing (EFSA GMO Panel, 2010a).

3.5.1. Environmental risk assessment

3.5.1.1. Persistence and invasiveness of the GM plant[64]

Cultivated soybean (*G. max*) is a species in the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop, generally unable to survive in the environment without appropriate management (Lu, 2005).

Occasional feral GM soybean plants may occur outside cultivation areas, but survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens and cold climatic conditions (OECD, 2000). Soybean can grow as volunteers and the presence of volunteers of *G. max* was occasionally reported in some areas of Italy where soybean is intensively cultivated (Celesti-Grapow et al., 2010). However, as for the same reasons mentioned above, soybean seeds usually do not survive during the winter (Owen, 2005).

Thus, the establishment and survival of feral and volunteer soybean in the EU is currently limited and transient.

It is unlikely that the intended traits of soybean MON 87751 will provide a selective advantage to soybean plants, except when they are infested by insect pests that are susceptible to the Cry1A.105 and/or Cry2Ab2 proteins. However, this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers it very unlikely that soybean MON 87751 will differ from conventional soybean hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable soybean MON 87751 seeds.

3.5.1.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA or through vertical gene flow via cross-pollination from feral plants originating from spilled seeds.

Plant-to-microorganism gene transfer[65]

Genomic DNA can be a component of food and feed products derived from soybean. It is well documented that such DNA becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, bacteria in the digestive tract of humans and animals, and in other environments, may be exposed to fragments of DNA, including the recombinant fraction of such DNA.

Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009).

The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments between to bacterial genomes is homologous recombination. This requires the presence of

[64] Dossier: Part II – Section 5.3.1.
[65] Dossier: Part II – Section 5.3.2.

at least two stretches of DNA sequences that are similar in the recombining DNA molecules. In the case of sequence identity with the transgene itself, recombination would result in gene replacement. In the case of identity with two or more regions flanking recombinant DNA, recombination could result in the insertion of additional DNA sequences in bacteria and thus confer the potential for new properties.

Soybean MON 87751 contains genetic elements originating or derived from bacteria (Section 3.2.1). These are: (1) a plant-optimised, synthetic *cry1A.105* gene derived from cry1Ab, cry1F and cry1Ac coding sequences from *B. thuringiensis*; and (2) a plant codon-optimised version of the *cry2Ab2* gene from *B. thuringiensis*.

Bioinformatic analyses of the inserted DNA demonstrated that the bacterial genes *cry1A.105*, and *cry2Ab2* did not provide sufficient sequence identity to facilitate homologous recombination and transfer of the recombinant DNA to bacteria in the receiving environments.

In addition to homology-based recombination processes, non-homologous (illegitimate) recombination that does not require the presence of DNA similarity between the recombining DNA molecules is theoretically possible. However, the transformation rates for illegitimate recombination were considered to be 10^{10}-fold lower than for homologous recombination (Hülter and Wackernagel, 2008; EFSA, 2009) and have not been detected for GM plants and bacteria, even in studies that have directly exposed bacteria to high concentrations of GM plant DNA (EFSA, 2009). Due to the plant codon-optimisation and poor expression of the recombinant *cry* genes connected to plant promoters as present on the recombinant DNA of soybean MON 87751, and the presence of natural variants of these genes in bacteria, it is highly unlikely that the recombinant cry-genes provide a selective advantage to bacterial recipients in the environment.

In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from soybean MON 87751 to bacteria. Given the nature of the recombinant DNA, the GMO panel identified no safety concern linked to an unlikely but theoretically possible horizontal gene transfer.

Plant-to-plant gene transfer[64]

The potential for occasional feral soybean MON 87751 plants originating from seed import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer were considered.

For plant-to-plant gene transfer to occur, imported GM soybean seeds need to germinate and develop into plants in areas containing sympatric wild relatives and/or cultivated soybean with synchronous flowering and environmental conditions favouring cross-pollination. It must be noted that most soybean MON 87751 seeds are processed in the countries of production or in ports of importation.

Vertical gene transfer from soybean (*G. max*) is limited to the species of the subgenus *Soja* to which *G. max* belongs to, as well as the wild relatives *G. soja* and *G. gracilis*. Although wild relatives exist elsewhere, no wild relatives of the subgenus Soja have been reported in Europe (Dorokhov et al., 2004; Lu, 2005). Therefore, vertical gene transfer from GM soybean is restricted to cultivated soybean (*G. max*).

Soybean is an annual, almost completely self-pollinating crop with a percentage of cross-pollination usually below 1% (OECD, 2000; Ray et al., 2003; Lu, 2005; Yoshimura et al., 2006; Abud et al., 2007), although natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions, such as favourable climate for pollination and an abundance of pollinators (Caviness, 1966; Gumisiriza and Rubaihayo, 1978; Kikuchi et al., 1993; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005).

The potential of spilled soybean seeds to establish, grow and produce pollen is extremely low and transient (see Section 3.5.1.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM soybean plants resulting from seed spillage, and weedy or cultivated soybean plants is also considered extremely low. Even if cross-pollination would occur, the GMO Panel is of the opinion that the likelihood of environmental effects as a consequence of the spread of genes from occasional feral GM soybean plants in Europe will not differ from that of conventional soybean varieties for the reasons given in Section 3.5.1.1.

3.5.1.3. Interactions of the GM plant with target organisms[66]

Taking the scope of application EFSA-GMO-NL-2014-121 into account (no cultivation), potential interactions of occasional feral soybean MON 87751 plants arising from seed import spills with target organisms are not considered a relevant issue by the GMO Panel.

[66] Dossier: Part II – Section 5.3.3.

3.5.1.4. Interactions of the GM plant with non-target organisms[67]

Given that environmental exposure of non-target organisms to spilled GM seeds or occasional feral GM soybean plants arising from spilled soybean MON 87751 seeds is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM soybean, potential interactions of soybean MON 87751 with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry proteins will not alter this conclusion.

3.5.1.5. Interactions with the abiotic environment and biogeochemical cycles[68]

Given that environmental exposure to spilled seeds or occasional feral soybean MON 87751 plants arising from seed import spills is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM soybean, potential interactions with the abiotic environment and biogeochemical cycles are not considered by the GMO Panel to raise any environmental safety concern.

3.5.2. Post-market environmental monitoring[69]

The objectives of a PMEM plan according to Annex VII of Directive 2001/18/EC are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific rationale of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from soybean MON 87751, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for soybean MON 87751 includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system newly established by EuropaBio for the collection of information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of soybean MON 87751. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

In the context of PMEM, the applicant should improve the literature searches according to the GMO Panel recommendations given in Section 3.1.

3.5.3. Conclusion on the environmental risk assessment and monitoring plan

The GMO Panel concludes that it is unlikely that soybean MON 87751 would differ from conventional soybean varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2014-121, interactions of occasional feral soybean MON 87751 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of horizontal gene transfer from soybean MON 87751 to bacteria does not indicate a safety concern. Therefore, considering the introduced traits, the outcome of the comparative analysis, and the routes and levels of exposure, the GMO Panel concludes that soybean MON 87751 would not raise safety concerns in the event of accidental release of viable GM soybean seeds into the environment.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean MON 87751.

[67] Dossier: Part II – Section 5.3.4.
[68] Dossier: Part II – Section 5.3.6.
[69] Dossier: Part II – Section 6.

4. Conclusions

The GMO Panel was asked to carry out a scientific assessment of soybean MON 88751 for import, processing, and food and feed uses in accordance with Regulation (EC) No 1829/2003.

The molecular characterisation data establish that soybean MON 87751 contains a single insert consisting of one copy of the *cry1A.105* and *cry2Ab2* expression cassettes. Bioinformatic analyses of the sequences encoding the newly expressed proteins and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA do not indicate significant similarities to toxins and allergens. The stability of the inserted DNA and of the introduced insect resistance trait is confirmed over several generations. The Cry1A.105 and Cry2Ab2 proteins were expressed and the methodology used to quantify their levels is considered adequate. The protein characterisation data comparing the structural, biochemical and functional properties of plant- and microbe-derived Cry1A.105 and Cry2Ab2 proteins indicate that these proteins are equivalent and the microbe-derived proteins can be used in the safety studies.

The GMO Panel concludes that none of the differences identified in the agronomic, phenotypic and compositional characteristics of soybean MON 87751 require further assessment regarding environmental and food and feed safety.

The GMO Panel does not identify safety concerns regarding the potential toxicity or allergenicity of the newly expressed Cry1A.105 and Cry2Ab2 proteins, and no evidence that the genetic modification might significantly change the overall allergenicity of soybean MON 87751 was found. The nutritional impact of soybean MON 87751-derived food and feed is expected to be the same as those derived from its conventional counterpart and non-GM commercial reference varieties. The GMO Panel concludes that soybean MON 87751-derived food and feed is nutritionally equivalent to and as safe as the conventional counterpart and the non-GM commercial reference varieties tested.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable seeds from soybean MON 87751 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of soybean MON 87751.

The literature searches did not identify any relevant publications on soybean MON 87751. In the context of PMEM, the applicant should improve the literature searches according to the GMO Panel recommendations.

In conclusion, the GMO Panel considers that soybean MON 88751, as described in this application, is as safe as its conventional counterpart and the tested non-GM soybean varieties with respect to potential effects on human and animal health and the environment.

Documentation requested and provided to EFSA

1) Letter from the Competent Authority of Netherlands received on 8 October 2014 concerning a request for placing on the market of genetically modified soybean MON 87751 submitted by Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003 (application reference EFSA-GMO-NL-2014-121).
2) Acknowledgement letter dated 15 October 2014 from EFSA to the Competent Authority of Netherlands.
3) Letter from EFSA to applicant dated 25 November 2014 requesting additional information under completeness check.
4) Letter from applicant to EFSA received on 22 December 2014 providing additional information under completeness check.
5) Letter from EFSA to applicant dated 22 January 2015 delivering the 'Statement of Validity' of application EFSA-GMO-NL-2014-121 for placing on the market of genetically modified soybean MON 87751 submitted by Monsanto Europe S.A./N.V. in accordance with Regulation (EC) No 1829/2003.
6) Letter from EURL to EFSA dated 26 January 2015 requesting to stop the clock.
7) Letter from EFSA to applicant dated 29 January 2015 requesting additional information and stopping the clock on behalf of the EURL-GMFF.
8) Letter from EURL to EFSA dated 11 March 2015 requesting to re-start the clock.
9) Letter from EFSA to applicant dated 16 March 2015 re-starting the clock on behalf of the EURL-GMFF.
10) Letter from EFSA to applicant dated 31 March 2015 requesting additional information and stopping the clock.

11) Letter from Applicant to EFSA received 20 May 2015 requesting clarification.
12) Letter from EFSA to Applicant dated 3 June 2015 providing clarification.
13) Letter from EFSA to applicant dated 5 June 2015 requesting additional information and maintaining the clock stopped.
14) Letter from applicant to EFSA received on 1 July 2015 providing additional information.
15) Letter from applicant to EFSA received on 15 July 2015 providing additional information.
16) Letter from EFSA to applicant dated 23 October 2015 re-starting the clock.
17) Letter from EFSA to applicant dated 2 December 2015 requesting additional information and stopping the clock.
18) Letter from applicant to EFSA received on 9 December 2015 providing additional information.
19) Letter from EFSA to applicant dated 23 December 2015 requesting additional information and maintaining the clock stopped.
20) Letter from applicant to EFSA received on 8 February 2016 providing additional information.
21) Letter from EFSA to applicant dated 11 February 2016 requesting additional information and maintaining the clock stopped.
22) Letter from EFSA to applicant dated 23 February 2016 requesting additional information and maintaining the clock stopped.
23) Letter from applicant to EFSA received on 24 February 2016 providing additional information and requesting clarification.
24) Letter from applicant to EFSA received on 14 March 2016 providing additional information.
25) Letter from EFSA to applicant dated 18 March 2016 requesting additional information and maintaining the clock stopped.
26) Letter from applicant to EFSA received on 22 March 2016 providing additional information.
27) Letter from applicant to EFSA received on 29 March 2016 providing additional information.
28) Letter from EFSA to applicant dated 6 April 2016 providing clarification.
29) Letter from applicant to EFSA received on 7 April 2016 requesting clarification.
30) Letter from applicant to EFSA received on 20 April 2016 providing spontaneous information.
31) Letter from EFSA to applicant dated 23 May 2016 providing clarification.
32) Letter from EFSA to applicant dated 23 May 2016 requesting additional information and maintaining the clock stopped.
33) Letter from EFSA to applicant dated 20 July 2016 requesting additional information and maintaining the clock stopped.
34) Letter from applicant to EFSA received on 21 July 2016 providing additional information.
35) Letter from applicant to EFSA received on 22 September 2016 providing additional information.
36) Letter from EFSA to applicant dated 23 September 2016 re-starting the clock from the 22 September 2016.
37) Letter from EFSA to applicant dated 7 December 2016 requesting additional information and stopping the clock.
38) Letter from applicant to EFSA received on 20 December 2016 providing additional information.
39) Letter from EFSA to applicant dated 21 December 2016 re-starting the clock from the 20 December 2016.
40) Letter from EFSA to applicant dated 23 December 2016 requesting additional information and stopping the clock.
41) Letter by email, from applicant to EFSA received on 23 December 2016 providing additional information.
42) Letter from EFSA to applicant dated 04 January 2017 re-starting the clock from the 23 December 2016.
43) Letter by e-mail only, from applicant to EFSA received on 2 February 2017 requesting clarification.
44) Letter from EFSA to applicant dated 3 February 2017 providing clarification.
45) Letter from EFSA to applicant dated 10 February 2017 requesting additional information and stopping the clock.

46) Letter from EFSA to applicant dated 8 March 2017 requesting additional information and maintaining the clock stopped.
47) Letter from EFSA to applicant dated 14 March 2017 requesting additional information and maintaining the clock stopped.
48) Letter from applicant to EFSA received on 11 April 2017 providing additional information.
49) Letter from applicant to EFSA received on 2 May 2017 providing spontaneous information.
50) Letter from EFSA to applicant dated 3 May 2017 requesting additional information and maintaining the clock stopped.
51) Letter from applicant to EFSA received on 15 May 2017 providing additional information.
52) Letter from applicant to EFSA received on 19 May 2017 providing additional information.
53) Letter from applicant to EFSA received on 31 May 2017 providing additional information.
54) Letter from EFSA to applicant dated 1 June 2017 re-starting the clock from the 31 May 2017.
55) Letter from EFSA to applicant dated 6 July 2017 requesting additional information and stopping the clock.
56) Letter from EFSA to applicant dated 2 August 2017 requesting additional information and maintaining the clock stopped.
57) Letter from applicant to EFSA received on 14 August 2017 providing additional information.
58) Letter from applicant to EFSA received on 22 August 2017 providing additional information.
59) Letter from EFSA to applicant dated 22 August 2017 re-starting the clock.
60) Letter from EFSA to applicant dated 25 September 2017 requesting additional information and stopping the clock.
61) Letter from applicant to EFSA received on 5 October 2017 providing spontaneous information.
62) Letter from EFSA to applicant dated 23 November 2017 requesting additional information and maintaining the clock stopped.
63) Letter from applicant to EFSA received on 23 November 2017 providing additional information.
64) Letter from EFSA to applicant dated 4 December 2017 requesting additional information and maintaining the clock stopped.
65) Letter from applicant to EFSA received on 4 December 2017 providing additional information.
66) Letter from applicant to EFSA received on 22 December 2017 providing spontaneous information.
67) Letter from applicant to EFSA received on 30 January 2018 providing additional information.
68) Letter from EFSA to applicant dated 31 January 2018 re-starting the clock from the 30 January 2018.
69) Letter from EFSA to applicant dated 7 March 2018 requesting additional information and stopping the clock.
70) Letter from applicant to EFSA received on 3 May 2018 providing additional information.
71) Letter from EFSA to applicant dated 3 May 2018 re-starting the clock.

References

Abud S, de Souza PIM, Vianna GR, Leonardecz E, Moreira CT, Faleiro FG, Júnior JN, Monteiro PMFO, Rech EL and Aragão FJL, 2007. Gene flow from transgenic to nontransgenic soybean plants in the Cerrado region of Brazil. Genetics and Molecular Research, 6, 445–452.

Ahrent DK and Caviness CE, 1994. Natural cross-pollination of 12 soybean cultivars in Arkansas. Crop Science, 34, 376–378.

Caviness CE, 1966. Estimates of natural cross-pollination in Jackson soybeans in Arkansas. Crop Science, 6, 211–212.

Celesti-Grapow L, Pretto F, Carli E and Blasi C (eds.)., 2010. Flora vascolare alloctona e invasiva delle regioni d'Italia. Casa Editrice Università La Sapienza, Rome. 208 pp.

Codex Alimentarius, 2009. Foods derived from modern biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome, Italy. 85 pp.

Dorokhov D, Ignatov A, Deineko E, Serjapin A, Ala A and Skryabin K, 2004. In: den Nijs HCM, Bartsch D, Sweet J (eds.). Introgression from Genetically Modified Plants into Wild Relatives. CAB International, Amsterdam, the Netherlands. pp. 151–161.

EFSA (European Food Safety Authority), 2008. Scientific Opinion of the Panel on Genetically Modified Organisms on application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of the insect-resistant genetically modified maize MON 89034, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. EFSA Journal 2008;6(12):909, 30 pp. https://doi.org/10.2903/j.efsa.2008.909

EFSA (European Food Safety Authority), 2009. Statement of EFSA on the consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants". EFSA Journal 2009;8(6):1108, 8 pp. https://doi.org/doi:10.2903/j.efsa.2009.1108

EFSA (European Food Safety Authority), 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010;8(6):1637, 90 pp. https://doi.org/doi:10.2903/j.efsa.2010.1637

EFSA (European Food Safety Authority), 2011. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/doi:10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2014. Explanatory statement for the applicability of the Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment. EFSA Journal;12(10):3871, 25 pp. https://doi.org/doi:10.2903/j.efsa.2014.3871

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010a. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879. 111 pp. https://doi.org/doi:10.2903/j.efsa.2010.1879

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010b. Statistical considerations for the safety evaluation of GMOs. EFSA Journal 2010;8(1):1250. 59 pp. https://doi.org/doi:10.2903/j.efsa.2010.1250

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010c. Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 2010;8(7):1700, 168 pp. https://doi.org/doi:10.2903/j.efsa.2010.1700

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011a. Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011;9(5):2150, 37 pp. https://doi.org/doi:10.2903/j.efsa.2011.2150

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011b. Guidance on the post-market environmental monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):2316, 40 pp. https://doi.org/doi:10.2903/j.efsa.2011.2316.

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2016a. Scientific Opinion on an application by Dow AgroSciences (EFSA-GMO-NL-2013-116) for placing on the market of genetically modified insect-resistant soybean DAS-81419-2 for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2016;14(12):4642, 23 pp. https://doi.org/10.2903/j.efsa.2016.4642

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2016b. Scientific opinion on application (EFSA-GMO-NL-2011-96) for the placing on the market of genetically modified insect-resistant and herbicide-tolerant cotton GHB119, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience AG. EFSA Journal 2016;14(10):4586, 27 pp. https://doi.org/10.2903/j.efsa.2016.4586

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2016c. Scientific Opinion on an application by Syngenta (EFSA-GMO-DE-2011-99) for the placing on the market of maize Bt11 x 59122 x MIR604 x 1507 x GA21 and twenty subcombinations, which have not been authorised previously independently of their origin, for food and feed uses, import and processing under Regulation (EC) No 1829/2003. EFSA Journal 2016;14(8):4567, 31 pp. https://doi.org/10.2903/j.efsa.2016.4567

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2017a. Scientific opinion on the assessment of genetically modified maize 1507 x 59122 x MON810 x NK603 and subcombinations, for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2011-92). EFSA Journal 2017;15(11):5000, 29 pp. https://doi.org/10.2903/j.efsa.2017.5000

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2017b. Scientific Opinion on application EFSAGMO-BE-2013-118 for authorisation of genetically modified maize MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 and subcombinations independently of their origin, for food and feed uses, import and processing submitted under Regulation (EC) No 1829/2003 by Monsanto Company. EFSA Journal 2017;15(8):4921, 32 pp. https://doi.org/10.2903/j.efsa.2017.4921

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2012. Scientific Opinion on Dietary Reference Values for protein. EFSA Journal 2012;10(2):2557, 66 pp. https://doi.org/doi:10.2903/j.efsa.2012.2557

EFSA Scientific Committee; 2011. EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA Journal 2011;9(12):2438, 21 pp. https://doi.org/doi:10.2903/j.efsa.2011.2438.

EFSA (European Food Safety Authority), Devos Y, Guajardo IM, Glanville J and Waigmann E, 2017. Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market. EFSA supporting publications 2017:EN-1207, 48 pp. https://doi.org/doi:10.2903/sp.efsa.2017.en-1207

Fernandez A, Mills EN, Lovik M, Spoek A, Germini A, Mikalsen A and Wal JM, 2013. Endogenous allergens and compositional analysis in the allergenicity assessment of genetically modified plants. Food and Chemical Toxicology, 62, 1–6.
Gumisiriza G and Rubaihayo PR, 1978. Factors that influence outcrossing in soybean. Zeitschrift für Acker- und Pflanzenbau/Journal of Agronomy and Crop Science, 147, 129–133.
Hülter N and Wackernagel W, 2008. Double illegitimate recombination events integrate DNA segments through two different mechanisms during natural transformation of *Acinetobacter baylyi*. Molecular Microbiology, 67, 984–995.
Huybrechts I, Sioen I, Boon PE, Ruprich J, Lafay L, Turrini A, Amiano P, Hirvonen T, De Neve M, Arcella D, Moschandreas J, Westerlund A, Ribas-Barba L, Hilbig A, Papoutsou S, Christensen T, Oltarzewski M, Virtanen S, Rehurkova I, Azpiri M, Sette S, Kersting M, Walkiewicz A, Serra-Majem L, Volatier JL, Trolle E, Tornaritis M, Busk L, Kafatos A, Fabiansson S, De Henauw S and Van Klaveren JD, 2011. Dietary exposure assessments for children in Europe (the EXPOCHI project): rationale, methods and design. Archives of Public Health, 69, 1169–1184.
Kikuchi A, Murata K, Tabuchi K and Sakai S, 1993. Inheritance of seed embryo color and investigation of degree of natural cross-pollination in soybeans. Breeding Science, 43(S2), 112.
Lecoq E, Holt K, Janssens J, Legris G, Pleysier A, Tinland B and Wandelt C, 2007. General surveillance: roles and responsibilities the industry view. Journal of Consumer Protection and Food Safety, 2(S1), 25–28.
Lu BR, 2005. Multidirectional gene flow among wild, weedy, and cultivated soybeans. In: Gressel J (ed.). Crop Ferality and Volunteerism, OECD + Taylor & Francis, Boca Raton, Florida. pp. 137–147.
Merten C, Ferrari P, Bakker M, Boss A, Hearty A, Leclercq C, Lindtner O, Tlustos C, Verger P, Volatier JL and Arcella D, 2011. Methodological characteristics of the national dietary surveys carried out in the European Union as included in the European Food Safety Authority (EFSA) Comprehensive European Food Consumption Database. Food Additives and Contaminants Part A, 28, 975–995.
OECD (Organisation for Economic Co-operation and Development), 2000. Consensus document on the biology of *Glycine max* (L.) *merr.* (soybean). Series on Harmonization of Regulatory Oversight in Biotechnology ENV/JM/MONO(2000)9, No. 15, 1–20.
OECD (Organisation for Economic Co-operation and Development), 2009. Guidance document on overview of residue chemistry studies. Series on testing and assessment No. 64. Series on pesticides No. 64. Organisation for Economic Co-operation and Development, ENV/JM/MONO(2009)31, Paris.
OECD (Organisation for Economic Co-operation and Development), 2012. Revised Consensus Document on Compositional Considerations for New Varieties of Soybean [*Glycine max* (L.) Merr.]: Key Food and Feed Nutrients, Anti-nutrients, Toxicants and Allergens. ENV/JM/MONO(2012)24.
Owen MDK, 2005. Maize and soybeans – controllable volunteerism without ferality? In: Gressel J (ed.). Crop Ferality and Volunteerism. OECD and Taylor & Francis, Boca Raton, Florida. pp. 149–165.
Ray JD, Kilen TC, Abel CA and Paris RL, 2003. Soybean natural cross-pollination rates under field conditions. Environmental Biosafety Research, 2, 133–138.
Selb R, Wal JM, Moreno FJ, Lovik M, Mills C, Hoffmann-Sommergruber K and Fernandez A, 2017. Assessment of endogenous allergenicity of genetically modified plants exemplified by soybean - where do we stand? Food and Chemical Toxicology, 101, 139–148.
USP, 1995. *Test solutions. Page 2053 in The National Formulary*. The United States Pharmacopeia, Washington, DC.
Vazquez RI, Moreno-Fierros L, Neri-Bazan L, de la Riva GA and Lopez-Revilla R, 1999. *Bacillus thuringiensis* Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scandinavian Journal of Immunology, 49, 578–584.
Windels P, Alcalde E, Lecoq E, Legris G, Pleysier A, Tinland B and Wandelt C, 2008. General surveillance for import and processing: the EuropaBio approach. Journal of Consumer Protection and Food Safety, 3(S2), 14–16.
Yoshimura Y, Matsuo K and Yasuda K, 2006. Gene flow from GM glyphosate-tolerant to conventional soybeans under field conditions in Japan. Environmental Biosafety Research, 5, 169–173.

Abbreviations

ADF	acid detergent fibre
ANOVA	analysis of variance
BSA	bovine serum albumin
bw	body weight
CTP	chloroplast transit peptide
DDE	daily dietary exposure
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
FOB	functional observational battery
GLP	Good Laboratory Practice
GM	genetically modified
GMO	genetically modified organisms
GMO Panel	EFSA Panel on Genetically Modified Organisms

IgE	immunoglobulin E
JSA	junction sequence analysis
LUC	large unstained cells
MCV	mean corpuscular volume
NDF	neutral detergent fibre
NGS	next generation sequencing
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SIF	simulated intestinal fluid